



NTP
National Toxicology Program

Report on the NTP Workshop: *Biomarkers for Toxicology Studies*

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NIEHS

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Charge of Meeting

- Experts from industry, academia and government called together for 2 days
 - Gail McCarver, Jon Mirsalis represented NTP BSC
- Charge: “to identify biomarkers and evaluate utility for inclusion in rodent toxicology studies to better characterize endpoints of environmentally induced human diseases or biological processes related to human disease etiology”
- Initial focus on heart, lung, lipid/carbohydrate markers; other tissues may be evaluated at a later date?



The “Ideal” Biomarker

- Method of analysis is appropriate to species being evaluated (e.g., human/rodent insulin assays have no homology)
- Sensitive, specific, predictive, efficient
- Bridges animal and human applications
- Non-invasive sampling (e.g., survival blood collection)
- Assay easy and rapidly performed
- Assay is reliable
- Assay is “cost worthy”



Approach to Workshop

- Presentations for each of the three tissues/endpoints
- Working groups tasked with identifying key biomarkers
- Went through worksheet for various biomarkers to assess utility, cost, etc.

Example:

Potential Biomarkers	Useful for predicting human disease or increased risk of disease from rodent study	Detects tissue injury or altered function	Methods for human samples applicable to rodent specimens	Other Special Concerns: e.g., specific time (s) for biomarker measurement; additional animals needed	Add to routine tox screen/ special studies/ or none
Cholesterol/ triglycerides	TC – yes TGs - yes	Yes	Yes	\$	Routine
Insulin	Yes	Yes	Yes, but must use rodent specific	\$\$\$\$, more sensitive indicator of IR than glucose	Routine
GSH (reduced glutathione)	Yes	Yes (oxidative stress)	Yes	Assay should be done on whole blood or RBCs (EDTA?)	Routine (not specific for lipid/CHO disorders)

- Answered series of general questions on top choices for inclusion



General Questions (*Example*)

- Is it applicable to rodents and humans? **Yes.** If not currently applied to rodents could it be? **Yes.**
- What is the disease process(es) evaluated (e.g., tissue injury, altered function, altered metabolism)? **Especially useful for markers of injury, inflammation, and other tissue response indicators.** Does it identify early or late events? **Largely useful for early events, but may be a good indicator of selected late events as well.**
- Is it sensitive, specific, and/or predictive of the disease process? **It could be all of these, depending on the panel of indicators chosen for investigation.** Could it be used to demonstrate a NOAEL? **Probably not.**
- What type of specimen/measurement is needed? **Whole lung or partial lung lavages are doable.** Is obtaining it noninvasive and easily accessible? **It is largely invasive in small animals, but easily accessible.** What is the appropriate time for sample or measurement collection? **Likely most informative at early time points.**
- Are there other special considerations with including it in NTP studies (e.g., extra animals needed)? **Depending on the number of other biomarkers assessed, extra animals may be necessary.**
- What technology is required? **Trivial.** Is it accurate, reproducible, and cost effective? **It can be. We recommend strongly that a SOP be developed to maximize these outcomes.**



Lung

- #1: Bronchialveolar Lavage Fluid Analysis
 - Useful for markers of inflammation
 - Might be too invasive for main study animals; require satellite group
- #2: Enhanced Histopathology
 - Primary focus on novel stains, immunohistochemistry (e.g, Ki-67)
 - Might need extra animals depending on what else you plan to do with lungs
- Other Markers With Promise
 - Gene Expression Analysis: Probably can't do all animals, but save (frozen) tissues for possible future analysis if lesions seen; as with other endpoints, might require additional animals depending on how much tissue you need or other uses
 - Imaging: Not quite ready for prime time, but has tremendous promise for the future



Heart

- #1: Troponins
 - Relatively inexpensive, non-invasive (blood), human correlates
 - Primarily an early event, may require early (days) interim sacrifice
- #2: B-type Natriuretic Protein (BNP)
 - More invasive (need RNA)
 - High negative predictivity, less so for a positive response
- #3: Ultrasound imaging
 - Non-invasive, good human correlates
 - Expensive, not high-throughput
- #4: α 2-macroglobulin (rat only)
 - Analogous to human C-reactive protein
 - Requires early (48 hr) sample time?



Lipids/Carbohydrates

- #1: Cholesterol/triglycerides
 - Widely available, human correlates, most other labs already do this
- #2: Insulin
 - Applicable to human, but need rodent specific methods
 - Recommended for routine use, but cost relatively high
- #3: Reduced Glutathione (GSH)
 - Good indicator of oxidative stress, low cost, reliable
 - Not specific to lipid disorders
- #4: Specialized Histopathology
 - Micro vs. macrovesicular fatty acid change
 - Inexpensive and physiologically meaningful
- Several other endpoints possible, but not for routine use
 - Body composition, hepatic CHO/lipid levels, SREBP-1,2



General Observations

- Many endpoints are most predictive at early timepoints (2-7 days)
 - Will require adding animals for an interim sacrifice group
- Some endpoints are invasive or compromise integrity of other endpoints (e.g., bronchoalveolar lavage) requiring extra satellite animals
- A number of biomarkers have human analogs already in use in human diagnosis (troponins, insulin, ultrasound, etc.) and these seem like “low hanging fruit” that are worth pursuing
- Some endpoints seem very promising, but will require significant training time, capital investment, etc. (e.g., microimaging, ultrasound, storage facilities for frozen tissues)
- Need to develop a decision tree approach to all categories
 - e.g., for cardiac, routinely do troponin, α 2-macroglobulin in the rat only, BNP in conjunction with ultrasound and markers of inflammation and necrosis. If a cardiotoxin suspected, add micro-CT.